AGING PREVENTING AGENT

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Abstract of **JP 5124980 (A)**

PURPOSE:To obtain an aging preventing agent consisting of a peroxidase and capable of prolonging human life and preventing aging by suppressing in vivo production of peroxilipids causing aging.
CONSTITUTION:The objective aging preventing agent is obtained by blending a peroxidase (preferably lactoperoxidase using cow milk as a feed source), as an active ingredient, preferably at a ratio of 0.1-5wt.%. This agent is preferably combinedly used with vitamin E as an antioxidant and/or lactoferrin. Furthermore, iron ion concentration in the aging agent is kept preferably <=50ppm in order to prevent the activity of the lacto peroxidase from the disturbance.

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(54)【発明の名称】　老化防止剤

(57)【要約】
【構成】　パーオキシダーゼ単独またはパーオキシダーゼと抗酸化剤とを有効成分とする老化防止剤。パーオキシダーゼにはラクトパーオキシダーゼが、抗酸化剤にはビタミンＥが用いられる。老化防止剤は、医薬、食品、飼料、化粧料の形態で用いられる。

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(54) AGING PREVENTING AGENT

(57)Abstract:
PURPOSE: To obtain an aging preventing agent consisting of a peroxidase and capable of prolonging human life and preventing aging by suppressing in vivo production of peroxilipids causing aging.
CONSTITUTION: The objective aging preventing agent is obtained by blending a peroxidase (preferably lactoperoxidase using cow milk as a feed source), as an active ingredient, preferably at a ratio of 0.1-5wt.%. This agent is preferably combinedly used with vitamin E as an antioxidant and/or lactoferrin. Furthermore, iron ion concentration in the aging agent is kept preferably ≤50ppm in order to prevent the activity of the lacto peroxidase from the disturbance.

CLAIMS

[Claim(s)]

[Claim 1]An antiaging agent making peroxidase into an active principle.

[Claim 2]The antiaging agent according to claim 1 whose peroxidase is lactoperoxidase.

[Claim 3]An antiaging agent making peroxidase, an anti-oxidant, and/or lactoferrin into an active principle.

[Claim 4]The antiaging agent according to claim 3 whose anti-oxidant is vitamin E or ascorbic acid.

[Claim 5]An antiaging agent whose peroxidase content is 0.1 to 5 % of the weight in either of claims 1-4.

[Claim 6]Iron ion concentration in an antiaging agent. The antiaging agent according to any one of claims 1 to 4 which is 50 ppm or less.

[Claim 7]The antiaging agent according to any one of claims 1 to 6 which is carrying out a gestalt of an oral composition.

[Claim 8]The antiaging agent according to any one of claims 1 to 6 which is carrying out a gestalt of external preparations.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application]This invention relates to the antiaging agent which makes peroxidase, especially lactoperoxidase an active principle.

[0002]

[Description of the Prior Art]Since a life is prolonged in advanced nations now and the natality rate is falling, social aging is progressing quickly. The biggest problem that will be faced when aging progresses is aging.

[0003]Aging is general terms, such as various phenomena produced in connection with aging, i.e., failure of eyesight, a memory disorder, an athletic ability fall, an immune-function fall, and hearing loss.

[0004]Although various factors as a cause of aging are shown, the DNA damage by the substance in a hyperoxidation state like active oxygen or a hydroxy radical is observed as a factor which determines aging speed. Therefore, a substance which prevents removal of such an oxidant or generation has the function to prevent aging.

[0005]As these substances, there are an enzyme or antioxidants, such as super-oxide dismutase, etc. Super-oxide dismutase and catalase are useful to remove a super oxide radical and hydrogen peroxide from in the living body, respectively. An antioxidant has the work which prevents aging by an oxidant.

[0006]The life of a substance like active oxygen, an oxygen radical, and a hydroxy radical is short. However, in the living body, these oxidation states are stored into peroxylipid. Peroxylipid has peroxide combination in the unsaturation fatty acid molecule, and becoming a cause of the secondary disease accompanying aging is pointed out.

[0007]The role with a transition metal important for generation of a hydroxy radical or peroxylipid is played. That is, as for iron ion, it is Janes et al. to promote generation of a malondialdehyde. It is reported by [Brain. Res, 246,113-119 (1982)], and, these days, he is Kobayashi et al. It is shown by [Agric. Biol. Chem., 54,69-76 (1990)] that hydrogen peroxide does damage to DNA under iron (II) and copper (II) ion existence. Therefore, it causes aging that such a transition metal exists superfluously in the living body.

[0008]To the aging prevention substance proposed until now, a hair-growing re-growth agent (JP,55-164616,A), There are foodstuffs (the No. Provisional-Publication-No. 61- 15423 gazette, JP,61-82744,A) containing the skin aging inhibitor (JP,56-115707,A), the plant extracted component, and bone marrow components by peptide content cosmetics, etc. As an aging prevention substance aiming at the prevention from peroxylipid generation, soybean saponin (the No. Provisional-Publication-No. 56- 73025 gazette) and an AZEPINO carboline derivative (the No. Provisional-Publication-No. 55- 65619 gazette) are proposed.

[0009]However, no these substances can control generation of the peroxylipid of

transition metal origin. On the other hand, he is Gutteridge et al. [Biochem. J, 199,259 (1981)] found out that the lactoferrin which is iron unity protein in milk controlled peroxylipid generation of an iron dependency.

[0010]

[Problem(s) to be Solved by the Invention]This invention persons prevented the generation of peroxylipid based on existence of the transition metal which is mainly concerned with iron in in the living body from the standpoint of becoming a cause by which generation of peroxylipid produces aging in this way, and looked for the substance which prevents aging. That is, there is SUBJECT of this invention in providing a new and effective antiaging agent.

[0011]This invention inquired [whether peroxidase, especially lactoperoxidase can control this generation and] paying attention to the generation of peroxylipid based on existence of the transition metal which is mainly concerned with iron such in the living body.

[0012]When this invention persons added various substances for foodstuffs first and examined peroxylipid generation depressor effect, they find out that the constituent which blended peroxidase, especially lactoperoxidase 0.1 to 5% of the weight can control generation of peroxylipid, and came to complete this invention.

[0013]In particular, when this invention persons retested the iron dependency peroxylipid generation inhibition test using lactoferrin, they examined whether peroxylipid generation depressant action would occur also about the enzyme in milk, and lactoperoxidase. It found out that lactoperoxidase, as a result, had peroxylipid generation depressor effect almost equivalent to lactoferrin.

[0014]An operation of lactoperoxidase is prevention of the hydroxy radical formation by disassembly of hydrogen peroxide. It is supposed in disassembly of hydrogen peroxide in the living body that catalase and glutathione peroxidase work (Toshihiko Osawa, monthly hood chemicals, and January, 1991 item P59-65). However, research detailed until now was not done about the antioxidant action of lactoperoxidase. This invention finds out that such lactoperoxidase has iron dependency peroxylipid generation depressant action in in the living body.

[0015]

[Means for Solving the Problem]This invention relates to an antiaging agent which makes peroxidase an active principle. This invention relates to an antiaging agent which makes peroxidase and an anti-oxidant an active principle.

[0016]Although myeloperoxydase, horseradish peroxidase, ARUSUROMAISE spar oxidase, etc. are used for peroxidase, use of a peroxylipid generation preventing effect and a point of safety to lactoperoxidase is desirable. Lactoperoxidase can be milk well prepared by mammalian. As a source of supply, although milk, such as a cow, a water buffalo, Homo sapiens, a swine, a sheep, a goat, and a horse, is raised, it is desirable from a field of quantity and quality to use cow's milk.

[0017]Lactoperoxidase is a publicly known substance, and in order to manufacture it, a method (JP,3-109400,A) of adsorbing lactoperoxidase and refining it using a publicly

known method, for example, a sulfonation carrier, can use it industrially advantageous.
[0018]Since iron and lactoperoxidase will synthesize lactoperoxidase and the operation will become weaker if iron ion exists, thing of iron ion concentration low as much as possible is desirable. When it comes to not less than 50 ppm, peroxylipid generation control ability by lactoperoxidase falls remarkably.
[0019]Lactoperoxidase can be added as an ingredient which has an aging prevention operation in a constituent of various gestalten. For example, it can mold by the ability to mix with a carrier of common use on pharmaceutical preparation, an excipient, etc., and can use as medicine or health food as a tablet, a granule, a capsule, drinkable preparations, etc. It mixes with a food composition, and it can also use as foodstuffs, it can mix with feed ingredients or a feed ingredient, and can use as feed and a feed. It can mix with an ingredient of not only taking-orally use such but cream, and can also use as external preparations, such as ointment for medicines, or facial cream. External preparations, such as oral compositions, such as not only pharmaceutical preparation with a common antiaging agent of this invention but such drugs, an eating-and-drinking article, feed, a feed, etc., cream, and ointment, etc. are called antiaging agent.
[0020]And although the amount of the peroxidase used changes with condition, sex, weights, etc., it is preferred to administer orally about 0.5-5 g of adult-man 1 sunny in 1 time thru/or several steps.
[0021]Germicidal treatment which does not have ******** in the enzyme activity of lactoperoxidase in manufacture of an antiaging agent is required. Although heating of sterilization in the mild state is desirable, if a method indicated by Japanese Patent Application No. No. 257010 [three to], i.e., a method of sterilizing in univalent salting in liquid beyond 0.1 mol, is used, a microorganism which prevents a fall of the enzyme activity of peroxidase and adheres can be ****(ed) efficiently.
[0022]It is still more desirable to blend with lactoperoxidase an anti-oxidant especially a lipophilicity antioxidant, for example, vitamin E, lactoferrin that carries out the chelate of the iron ion, etc. As an anti-oxidant, although there are erythorbic acid, sodium erythorbate, dl-**-tocopherol (vitamin E), etc., these can also be used. However, it is useful especially from doing so an antioxidant effect where especially vitamin E exists naturally and which does not have toxicity. The amount used is among a constituent. 0.001 to 1% is desirable.
[0023]It is effective in removing iron ion in which lactoferrin carries out the catalyst of the radical formation to it being thought that lactoperoxidase is effective in removal of hydrogen peroxide by which it was generated in the living body. Therefore, by making both lactoperoxidase and lactoferrin intermingled, an operation mechanism of an antiaging agent spreads and, as a result, an effect can be extensively demonstrated to various peroxylipid generation factors. Also the amount of lactoferrin used It is desirable that it is 0.1 to 5 % of the weight.
[0024]In addition to lactoperoxidase and lactoferrin, generation of peroxylipid in the living body can be synergistically controlled by using vitamin E together. It is good to

combine with other food materials with few iron contents preferably, in this invention, so that action expression of lactoperoxidase may not be blocked.

[0025]By the following examples of an examination, this invention persons determined an addition of lactoperoxidase.

[0026][The example 1 of an examination]

A physiological saline (pH 8.2) which contains 5-ml 20-m HEPESU (N-[2-hydroxyethyl] piperazine N'-[2-ethane-sulfonic-acid]) in a liposome kit (yolk lecithin origin) of a control sigma company of iron dependency peroxylipid generation by lactoperoxidase is added, It ultrasonicated and liposome was prepared. $FeNH_4(SO^4)_2$ solution 100mul of liposome 200microl and 80 muM, ascorbic acid 100microl of 300microM, and lactoperoxidase solution 100microl were put into a test tube with a lid, and a physiological saline adjusted the whole quantity to 1 ml further. It is the TBA method about peroxylipid which produced mixed liquor after a 1-hour incubation at 37 **. It measured by [Buege and J.A. and Aust. S.T.D. MethodsEnzymol., 52,302-310 (1978)].peroxylipid concentration -- a malondialdehyde (MDA) -- a considerable quantity was used and it displayed.

[0027]A result is shown in Table 1.

[Table 1]

---. lactoperoxidase concentration Peroxylipid generated amount control rate (final concentration and mg/ml) (nmol) (%). ---. 1.0 0.22 100.0 0.5 0.28 90.6 0.1 0.70 25.0 0.01 0.80 9.4 0 0.86 0. Control 0.22 of --- --- iron-ascorbic acid non-** (-) ---

[0028]0.1mg/ml of lactoperoxidase controlled peroxylipid generation by iron-ascorbic acid 25% so that it might see in this table. 1mg/ml of lactoperoxidase was controlled thoroughly.

[0029][The example 2 of an examination]

It gave the feed of the presentation shown in the peroxylipid generation control table 2 in blood in a lactoperoxidase feed rat to five 4-week old SD system male rats (CLEA Japan) at a time, heart blood collecting was performed under anesthesia after six-week breeding, and the peroxylipid concentration in blood was measured by the TBA method. A result is shown in Table 3. By the group which added lactoperoxidase 1% or more in the test meal, the remarkable fall of the peroxylipid concentration in blood was seen so that it might see in Table 3.

[0030]

[Table 2]

---. mark semi- -- Meal Trial ** A meal. ---. Casein 25% 25% corn oil 55 salts mixture *4 Four Vitamin mixture **1 1 choline-chloride 0.2 0.2 Cow lactoperoxidase -- 0.5, 1, 2, 4 cane sugars With a cane sugar, the whole quantity. --- --- * mineral mixture MM-2 which made the whole quantity 100% and

100% carried out with the cane sugar [Ebihara et al. J. Nutr.109-2106 (1979)]
** Harper's mixture [Harper, J. Nutr., 68, and 405 (1959)]
[0031]
[Table 3]
The amount of ---------- ---------- lactoperoxidase Peroxylipid concentration in blood (average value of five animals)
(%) (nmol/ml) ---------- -- 0 1.86 0.5 1.68 1 1.24 2 1.30 4 1.32 ----------

[0032]Iron content in a test meal It was 38 ppm. From the examples 1 and 2 of an examination, it is a lactoperoxidase addition. It was judged that 0.1 to 5% was suitable.
[0033]Next, the example of this invention is shown and this invention is explained still more concretely.
[0034]
[Work example 1]
Lactoperoxidase content antiaging agent (1) powdered-food powdered skim milk The cow lactoperoxidase powder 20g and the oligosaccharide powder 20g containing galactosyl lactose 35% were mixed to 960 g, and powdered food was prepared. The iron content was 5 ppm.
[0035](2) adding water to the material mix mixed with the compounding ratio shown in the drink table 4 -- the whole quantity [ ] -- being referred to as 100 l. -- this -- a jacket type tank -- after 65 ** and the heat sterilization during 30 minutes, and plastic sterile blow bottle [ ] -- it filled up with 160 ml at a time. The iron content was 1 ppm.
[0036]
[Table 4]
----------. sucrose 11-kg scent charge [ ] -- 100g frozen concentrated juice (Bx45) -- 12l. cow lactoperoxidase 600g dl-**-tocopherol (1%, alcohol solution) 50ml ----------

[0037](3) It is powdered skim milk to the yogurt water 22.1g. Dissolve 3.6 g and at 95 ** After 30-minute heat sterilization maintenance, It cools at 37 ** and he is a mixed starter of commercial L.bulgaricus and S.thermophilus (all are Hansen). 0.3g was inoculated and it cultivated at 37 ** for 6 hours. To this culture, it added lactoperoxidase and 2g of lactoferrin at a time, respectively, and agitated and cooled, and the lactic starter for yogurt preparation was obtained.
[0038]Next, milk fat 0.05 g of vitamin E is added to 970 g of fresh milk (commercial cow's milk) standardized to 3.5%, It cooled at 37 ** after heat sterilization maintenance for 10 minutes at 90 **, and 30g of lactic starters prepared previously were inoculated, it cooled promptly after culture to 0.85% of lactic acid acidity at 37 ** after filling up a cup, and plain yogurt was prepared. The iron content of yogurt was 1.2 ppm.

[0039](4) Mixed emulsification of the presentation shown in Table 5 below facial cream was carried out, and cream was prepared.
[Table 5]

Polyhydric alcohol fatty acid ester 10 g Liquid paraffin . 10g 1,3-butylene glycol 5 g. Stearic acid 5 g Glycerine fatty acid ester . 5g polyethylene glycol fatty acid ester [0.1 g Lactoferrin 100 mg lactoperoxidase 100 mg deionized water / Whole quantity To 100 g, Adjustment .] 2 g Behenyl alcohol 1 g Methylparaben 0.1 g Butylparaben

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[0040]
[Work example 2]
SD system rat (4-week old male) of five groups was made to carry out free ingestion of the drink prepared by product administration examination ** example 1-(2). Feed gave commercial feed (CLEA Japan CE-2). The peroxylipid concentration in blood of six weeks after was measured according to the example 2 of an examination. The result is shown in Table 6.
[0041]
[Table 6]

** Charge peroxylipid concentration in blood (nmol/ml) water 1.76 -- examination drink 1.30

[0042]It was checked that the drink of example 1-(2) is effective in reducing the peroxylipid in blood.
[0043]
[Work example 3]
The powdered food manufactured by product administration examination ** example 1-(1) was blended with feed as shown in Table 7, the BALB/C system mouse (5-week old male) of ten groups was given, free ingestion of water and the feed was carried out, it bred over a long period of time, and the life was measured.
[0044]
[Table 7]

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mark (weight section) semi- -- Meal Trial ** meal (weight section).

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powdered-skim-milk 30 - oligosaccharide powder 0.6 - example 1- powder - 30 corn-oil 3 of (1) 3 salts mixture (MM-2) -- 4 Vitamin mixture 1 of four harbors 1 choline-chloride 0.2 0.2 cane sugars a cane sugar -- the whole quantity The whole quantity was set to 100 with the cane sugar. It was referred to as 100.

[0045]This test result is shown in Table 8. By the test meal administration group, the

life was extended remarkably and it was checked that there is the life extension effect.
[Table 8]

---------- life expectancy ---------- - mark semi- -- Meal 596 days Trial ** Meal 625-day ----------

[0046]
[Effect of the Invention]Since the antiaging agent of this invention does so the effect which controls generation of the peroxylipid leading to aging in the living body, is made to extend a life, and carries out aging prevention, it is useful as advanced functional composition.